PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, C07K 14/47, 1/14	A1	(11) International Publication Number: WO 96/18734
		(43) International Publication Date: 20 June 1996 (20.06.96)

(21) International Application Number: PCT/EP95/04797

(22) International Filing Date: 6 December 1995 (06.12.95)

(30) Priority Data:
94120019.8 16 December 1994 (16.12.94) EP
(34) Countries for which the regional or international application was filed: AT et al.

(71) Applicant *(for all designated States except US)*: CIBA-GEIGY AG [CH/CH]; Klybeckstrasse 141, CH-4002 Basle (CH).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: VAN OOSTRUM, Jan [CH/CH]; Mühleweg 11, CH-4112 Flüh (CH). ASSELBERGS, Fredericus, Alphonsus, Maria [NL/CH]; Rainallee 88/3, CH-4125 Riehen (CH). LEIST, Christian [CH/CH]; Hofackerstrasse 7, CH-5073 Gipf-Oberfrick (CH). GRÜTTER, Markus [CH/CH]; Nettenbergstrasse 1, CH-4146 Hochwald (CH).

(74) Common Representative: CIBA-GEIGY AG; Patentabteilung, Klybeckstrasse 141, CH-4002 Basle (CH).

(81) Designated States: AL, AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PRODUCTION OF RECOMBINANT SECRETORY COMPONENT

(57) Abstract

The current invention relates to a recombinant secretory component (rSC) obtainable from a Chinese Hamster Ovary (CHO) cell that can be crystallized and, hence, is susceptible to systematic studies of its 3-dimensional structure. The crystallized form may be used directly or indirectly (e.g. *via* the derived structure) for lead finding, screening and binding studies.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

PRODUCTION OF RECOMBINANT SECRETORY COMPONENT

The current invention relates to a new form of a recombinant secretory component (rSC), obtainable from a recombinant Chinese Hamster Ovary (CHO) cell line, that can be crystallized and, hence, is susceptible to systematic studies of its 3-dimensional structure. The crystallisable form may be used directly (e.g. to stabilize immunoglobulins) or indirectly (e.g. *via* the derived structure) for lead finding, screening and binding studies.

In vivo, the secretory component (SC) is synthesized as the extracellular part of an integral membrane glycoprotein of specific cells which are responsible for the transport of polymeric immunoglobulin (Ig) to external mucosal surfaces. Such cells include epithelial cells found in several tissues including those in the lining of the respiratory, gastrointestinal, biliary and urogenital tracts and in the salivary, lacrimal, and mammalian gland and also hepatocytes. This membrane protein is termed polymeric immunogobulin receptor (pIgR) and specifically binds polymeric IgA (poly-IgA) and IgM (poly-IgM) on the basal side of the cells. During transport of the pIgR/poly-Ig complex from the basal to the apical (extracorporal) side of the cell, the pIgR is cleaved thus forming the SC. Most of the SC is released from the cell as part of the SC/poly-Ig complex, but also free SC is found in external secretions. In the SC/poly-Ig complex, SC is thought to stabilize the quaternary structure of poly-Ig and to increase resistance of the complex to various proteolytic enzymes. This resistance, for example to digestive proteases, is an important if not essential prerequisite for the protective function of secretory immunoglobulins.

Accordingly, SC usually occurs in three molecular forms:
- as a membrane protein on the surface of epithelial cells and hepatocytes;
- as a component chain of secretory IgA and soluble IgM; and
- as a free glycoprotein in several external secretions.

Based on cDNA (SEQ ID NO 1), the deduced amino acid sequence of human pIgR (SEQ ID NO 2) has a length of 764 residues and shows overall similarity of 56% and 64% with the rabbit and rat counterparts, respectively (Krajci *et al.*, Human Genetics (1991), **87**, 642-648). Free SC isolated from human milk is a 78-kDa single chain glycoprotein with a content of up to 20% carbohydrate. The primary sequence analysis indicates that SC consists of five domains, each approximately 100 amino acids in length. These domains are homologous in size and sequence to the domains characteristic of the immunoglobulin superfamily of proteins.

As described above, pIgR mediates the transport of IgA and IgM into mucosal secretions. These mucosal antibodies are of enormous importance in the immune response. They are capable of inactivating pathogens that have not yet entered the body and those that do not enter the body but which cause disease by secreting toxins which are taken up by the body. Accordingly, antagonists and, preferable, agonists of SC are of great pharmaceutical value for the modulation of mucosal immune response.

For effective drug design it is important to know the 3-dimensional structure of a receptor. Despite various efforts, neither the pIgR nor native SC have been crystallized successfully yet.

The glycosylation pattern of a recombinant glycoprotein, such as rSC, is determined by certain ill-understood determinants in the amino acid sequence of the protein, and by the availability of competing processing enzyme activities which determine the final structure of the glycans. Thus different glycosylation variants, termed glycoforms, of the same polypeptide can be produced by different cell lines, depending on the distribution of competing oligosaccharide processing enzyme activities in these cell lines. The type of glycosylation of the glycoprotein influences functional and structural parameters of the protein. For example, glycosylation often enhances stability of the polypeptide in the presence of proteolytic enzymes. On the other hand, substantial glycosylation, as especially in the case of SC, often interferes with attempts to crystallize the protein.

Recombinant glycoproteins expressed in CHO cells are known to contain carbohydrate chains terminally substituted with sialic acids. The most prominent sialic acids, α 2-3-linked N,O-acetylneuraminic acids (NeuAc) and α 2-3-linked N-glycolylneuraminic acid (NeuGc), are present in a ratio of about of about 97:3 (Hokke *et al.*, FEBS Lett. (1990), **275**, 9-14). The occurrence of N-acetylneuraminate mono-oxygenase (EC 1.14.99.18) activity, responsible for conversion of CMP-Neu5Ac into CMP-Neu5GC acting as precursor for incorporation of Neu5Gc in the glycoprotein, has not been demonstrated to come to expression in normal adult human tissue. Extensive studies have shown that when normal adult humans are exposed to sera of animal species, immunogenic responses may occur. The so-called Hanganutziu-Deicher (serum sickness) antibodies are directed towards glycoconjugates containing terminal Neu5Gc, α2-3-linked to β-Gal. Surprisingly we found that CHO SSF 3 cells incorporates NeuGc in the carbohydrate chains of rSC in amounts much lower than thus far observed for a typical glycoprotein produced by any other CHO cells. The ratio of NeuGc for rSC produced in CHO SFF3 cells was below 0.25% of total

sialic acid whereas a typical recombinant glycoprotein produced in CHO cells contains about 3%. The reduced content in NeuGc, usually not present in human glycoproteins, makes rSC produced by CHO SSF 3 cells more human-like and thus less antigenic and safer for human applications.

A further surprising fact is, that it is possible to produce glycoforms of recombinant SC (rSC), which are both active in binding polymeric immunoglobulin and can be crystallized. The inventive glycosylated rSC can be crystallized, e.g., by the 'hanging drop method' and provides an ideal starting point for the evaluation of the 3-dimensional structure of SC and facilitate the search for SC antagonists and, preferably, SC agonists including muteins of the SC polypeptide itself.

Detailed description of the invention

The current invention relates to a recombinant secretory component (rSC) or a functional fragment thereof, obtainable by a process comprising culturing a CHO SSF 3 cell transfected with a vector comprising a DNA coding for said secretory component or a functional fragment thereof, and isolating the expressed protein from the culture medium.

The rSC as defined above has, for example, a content of N-glycolylneuraminic acid (NeuGc) that is below 0.5% in respect to total sialic acid. Hence, the inventive rSC exhibit, for example, decreased antigenicity in human.

The inventive process preferably comprises the following steps:

a) constructing a vector capable of expressing the secretory component or a functional fragment thereof;
b) transfecting a CHO SSF 3 cell with said vector;
c) culturing the transfected cells; and
d) isolating the secretory component or a fragment thereof from the culture medium.

Secretory component (SC)

The pIgR is a receptor capable to bind to poly-Ig, especially IgA and IgM. Human pIgR usually has nucleotide sequence as depicted in SEQ ID NO 1, and an amino acid sequence basically as depicted in SEQ ID NO 2. The pIgR is cleaved during transport of the pIgR/poly-Ig complex from the basal to the apical (extracorporal) side of the cell thus forming the SC. A functional fragment of rSC is a derivative of pIgR that has one or more

amino acid deletions or modifications, that is capable of binding to poly-Ig, especially poly-IgA, and/or that reacts in the presence of antagonists and, preferable, agonists comparable to natural SC. A preferred fragment is, for example, a soluble fragment that lacks a membrane anchoring domain that resides, e.g., between amino acids 639-661 of the pIgR, and/or the intracellular C-terminal domain between amino acids 662-764. Especially preferred are fragments as depicted in SEQ ID NO 3 and SEQ ID NO 4.

The DNA fragments coding for said rSC or the fragment thereof may be modified, e.g., in so far as to adapt the codons to the preferred codon usage of the host, or the DNA sequence immediately in front or behind of the coding sequence may be modified in order to enhance the transcription, stabilize the mRNA produced, or to ease the genetic modifications, e.g., by an insertion of restriction sites.

Plasmids

The DNA coding for the secretory component or a functional fragment thereof, as described above, usually is comprised in a polypeptide expression cassette capable of expressing said DNA. In a preferred expression cassette according to the invention, a promoter is operably linked to said DNA that is transcribed under the control of this promoter, and to a terminator.

The promoter can be of almost any origin. It is for example possible to use a tightly regulated promoter or the promoter that is naturally adjacent to said DNA. Preferred are promoter that are active in CHO cells like viral promoters such as the 'early' promoter of SV40, the immediate early promoter of a cytomegalovirus (mouse, simian or human), or cellular promoters such the promoter of the β-actin gene, the metallothionein gene, or the heat shock genes. Especially preferred is the SV40 early promoter for the NEO gene, the mouse or human cytomegalovirus immediately early promoter for the SC and the human adenovirus type 2 promoter for the DHFR gene.

A DNA sequence containing the transcription termination signals is preferably the 3' flanking sequence of a gene which contains proper signals for transcription termination and polyadenylation for the desired host. Suitable signals are, for example, the polyadenylation signal of cellular genes such as the human growth hormone or the rabbit β-globin gene, or of viral genes the as those of the SV40 'early' and 'late' genes.

The plasmids may also contain fragments of DNA that increase the stability of the plasmid in the desired host or that ease the integration of the plasmid DNA or the essential part thereof into the chromosome of the desired host. Examples for suitable DNA fragments are the long terminal repeats of retroviruses, in case the recombinant genes are to be transferred as retrovirus particles, viral origins of replication, such as SV40, EBV, AAV, *vaccinia*, papillomavirus, Semliki forest virus etc., or DNA segments containing recognition sites for site-specific recombinases such as CRE and FLP.

The promoter, the DNA sequence coding for the secretory component or a functional fragment thereof and the DNA sequence containing transcription termination signals are operably linked to each other, i.e. they are juxtaposed in such a manner that their normal functions are maintained. The array is such that the promoter effects proper expression of the SC gene and the transcription termination signals effect proper termination of transcription and polyadenylation. The junction of these sequences may, for example, be effected by means of synthetic oligodeoxynucleotide linkers carrying the recognition sequence of a specific endonuclease.

The expression cassettes according to the invention may be maintained in the desired host in form of a stable episome or plasmid or as part of the chromosome, wherein the latter case is preferred.

However, it is likewise possible that the expression plasmids according to the invention include one or more, especially one or two, selective genetic markers for the host used for the construction, amplification and test of the plasmid, such a marker and an origin of replication for a bacterial host, especially *Escherichia coli*.

As to the selective gene markers, any marker gene can be used which facilitates the selection for transformants due to the phenotypic expression of the marker gene. Suitable markers are, for example, those expressing resistance to an antibiotic or another antimetabolite or, in the case of auxotrophic host mutants, genes which complement host lesions. Corresponding genes confer, for example, resistance to the antibiotics tetracyclin, ampicillin, G418, hygromycin, puromycin or bleomycin or provide for prototrophy in an (conditionally) auxotrophic mutant, for example the thymidine kinase (TK) gene, dihydrofolate reductase (DHFR) gene and the *E. coli* gpt, HisD or Trp genes. These selectable marker genes can also be provided by cotransfection of the expression gene with a physically unlinked selection gene. After such a cotransfection the enzymatic

machinery will with great probability cointegrate the two gene sets such that elimination of untransfected cells is possible.

As the amplification of the expression plasmids is usually done in a prokaryote, such as *E. coli.*, a prokaryote, e.g. *E. coli*, genetic marker and a prokaryote, e.g. *E. coli*, replication origin are included advantageously. These can be obtained from corresponding prokaryotic plasmids, for example *E. coli* plasmids, such as pBR322, pTZ18R, or a pUC plasmid, for example pUC18 or pUC19, which contain both prokaryotic, e.g. *E. coli*, replication origin and genetic marker conferring resistance to antibiotics, such as ampicillin and tetracyclin.

Apart from the polypeptide expression cassette, replication origin(s) and genetic marker(s) the expression plasmids according to the invention can contain optionally additional expression cassettes, such as 1 to 3 additional polypeptide expression cassettes, which may be the same or different.

Examples for suitable vectors are mammalian cell expression vectors based, for example, on pEUK-C1 (Clontech Inc., Palo Alto, California, USA), pcDNAIneo (Invitrogen Corp. San Diego California, USA) pCGA28 (Asselbergs *et al.* Fibrinolysis (1993), 7, 1-14) or pCGA93D-PPREN (Asselbergs *et al.*, Biotech. (1994), 32, 191-202).

The expression plasmids according to the invention are prepared by methods known in the art, for example by linking the polypeptide expression cassette, the DNA fragments containing selective genetic markers for the host used in the test and optionally for a bacterial host, the origin(s) of replication, and the optionally additional polypeptide expression cassettes in the predetermined order using conventional chemical or biological *in vitro* synthesis procedures. Preferentially, the plasmids are constructed and prepared using recombinant DNA techniques. For the preparation by recombinant DNA techniques suitable DNA fragments are ligated *in vitro* in conventional manner. The ligation mixture is then transformed into a suitable prokaryotic or eukaryotic host depending on the nature of the regulatory elements used, and a transformant containing the desired vector is selected according to conventional procedures. The plasmids can be multiplicated by means of the transformed hosts and can be isolated in conventional manner. The choice of the host depends on the regulatory sequences located on the vector. For the construction and multiplication of the vector a prokaryotic host, e.g., *E. coli*, is preferred.

Hosts, transfection and culturing

A suitable host for the production of rSC is a CHO SSF 3 cell (Gandor, C.R. (1993) Establishment and characterization of growth-factor-prototrophic Chinese hamster ovary (CHO) cell lines for the production of recombinant proteins, Zürich: Dissertation Nr 10087, Swiss Federal Institute of Technology.) or a cell that is derived therefrom and produces the same glycoforms of rSC.

The suitable host, as defined above, can be transfected by the standard methods in genetic engineering, as for example with the aid of cationic lipid vesicles, electroporation or particle gun. To increase the amount of rSC produced, it is advantageous to use a high copy plasmid or the plasmid DNA is integrated into the genome in several copies. The latter can be achieved, for example, *via* an amplification with methotrexate as described for example in (Asselbergs *et al.* J. Biotechnol. (1994), **32**, 191-202,; Asselbergs *et al.* J. Biotechnol. (1992), **23**, 143-151; Asselbergs *et al.* J. Mol. Biol. (1986), **189**, 401-411 and Kaufman *et al.* Mol. Cell Biol. (1985.), **5**, 1750-1759,).

The modified CHO SSF 3 cell can be cultured by standard methods in cell culture. In a preferred embodiment of the invention the cells are cultured in a serum-free medium and more preferred in a serum- and protein-free medium.

Surprisingly, it has been found, that the addition of Pluronic® to the culture medium has a productivity enhancing effect. Although, the mean doubling time is about 20% lower than in the absence of Pluronic® the daily yield of rSC is several times higher. This selective effect on the cell specific productivity is surprising and has not been observed yet. The amount of Pluronic®, especially Pluronic F-68®, added to the culture medium, is preferably about 0.005 to 0.5% (w/v) and more preferred 0.01 to 0.1% (w/v).

Isolation

The rSC produced by the inventive method is secreted predominantly in to the culture medium. It can be isolated therefrom by conventional means. During the isolation conventional additives like protein stabilizers, inhibitors of proteinases and the like may be added. For example, the first step consists usually in separating the cells from the culture fluid by means of centrifugation or filtration. In the presence of additional proteins and impurities, the resulting supernatant can be enriched for rSC. Representative purification schemes include, e.g., treatment with polyethyleneimine as to remove most of the non-

proteinaceous material, and precipitation of proteins by saturating the solution with ammonium sulfate or the like, ultrafiltration, diafiltration, gel electrophoresis, carrier-free electrophoresis, chromatographic processes such as ion exchange chromatography, size exclusion chromatography, partition chromatography, affinity chromatography, HPLC, reverse phase HPLC, treatment with Sephadex®, dialysis, or by other processes, especially those known from the literature. Those skilled in the art would appreciate that a combination of purification schemes can be used. In general, only a few purification steps are required in order to obtain a rSC product which is essentially free of contaminants.

Crystallization

A further embodiment of the invention is a method for the crystallization of the secretory component (SC) according to the invention comprising placing a solution of said secretory component in a vessel containing a precipitating agent buffer, wherein the solution and the buffer are separated. Common methods and details for the crystallization of protein according to the 'hanging drop' method are, for example, described in Mc Pherson, A (1982): Preparation and Analysis of protein crystals. John Wiley and Sons, NY.

To crytallize the protein the solution of the secretory component or the functional fragment thereof can contain a precipitating agent buffer. It is, for example, preferred to mix the solution comprising the inventive rSC and the precipitating agent buffer in an amount of 1:2 to 2:1 or, preferred, in about equal amounts.

The precipitating agent buffer usually contains in addition to the compounds used to establish a certain pH, one or more hygroscopic compounds and preservatives. Examples for suitable ingredients are NaN_3, Na-citrate, HEPES, ammonium phosphate, and/or Li_2SO_4. Preferred buffers comprise for example a mixture of Na-citrate, ammonium phosphate and NaN_3; or a mixture of HEPES, Li_2SO_4 and NaN_3.

In a further preferred method the solution comprising rSC is placed in a hanging manner over the precipitating agent buffer (hanging drop method).

The crystallization is carried out preferred at temperatures from 3°C to 30°C, more preferred from 5°C to 25°C, and especially preferred at room temperature.

Use of the isolated rSC

The isolated and crystallized rSC as described above can be used to identify the 3-dimensional structure of the whole protein or at least of the areas responsible for binding and secretion of poly-Ig. Conventional methods for the identification of the 3-dimensional structure are, for example, X-ray studies or NMR studies. The data received with these or comparable methods may be used directly or indirectly for the identification of antagonists or, preferably, agonists of the rSC mediated IgA transport. A commonly used method in this respect is, for example, computer aided drug design or molecular modeling.

The highly enriched rSC also may be used directly for binding studies and in the screening of compounds for their ability to influence poly-Ig binding. For these tests rSC according to the invention may be, for example, immobilized on a solid carrier like a micro titer plate or on beads; or may bear one or more identifiable maker like biotin or a radioactive, fluorescent or chemoluminescent group.

A further embodiment of the invention concerns the antagonist or, preferably, agonist identified with the inventive rSC, or with the aid of the 3-dimensional structure derived therefrom, for use in a method of treatment.

The inventive rSC has valuable pharmaceutical properties because of its lower immunogenicity in respect to previously known SC. This lower immunogenicity is based, e.g., on a low content of NeuGc (0.25% or less of the total sialic acid content). Accordingly, a further embodiment of the invention concerns the use of the inventive rSC as defined above in a method of treatment, e.g., in the stabilization of poly Ig, especially poly IgA.

EXAMPLES:

The following examples illustrate the invention and should not be construed as a limitation thereof.

Standard methods in genetic engineering like cleavage with restriction enzymes, ligations, transformation and annealing are carried out essentially as described in *Sambrook et al.*, Molecular Cloning: A laboratory manual, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY, 1989.

Example 1: human polymeric immunoglobuline receptor cDNA

The cDNA sequence of the human polymeric immunoglobuline receptor (pIgR, SEQ ID NO:1) is known (Krajci *et al.*, Biochem. Biophys. Res. Commun. (1989), **158**, 783-789; Krajci *et al.*, Hum. Genet. (1991), **87**, 642-648; Piskurich *et al.*, Mol. Immunol. (1993), **30**, 413-421; SEQ ID NO 1). Such cDNA can be generated using standard methods in genetic engineering, e.g., by reverse transcription of mRNA from samples of tissue expressing pIgR (Krajci *et al.*, 1989, opus cit.) or from a publicly available cell line such as HT29 (ATCC HTC-38, Piskurich *et al.*, Mol. Immunol. (1993) **30**, 413 -421). The cloned cDNA can be identified by hybridization with PCR fragments generated from the cDNA mixture with primers designed using the cDNA sequences in the public domain.

The cDNA is cloned in a plasmid vector, which can be multiplied in *E. coli*. Plasmid DNA is prepared according conventional procedures (*Sambrook et al.*, Molecular Cloning: A laboratory manual, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY, 1989) and the nucleotide sequence of the cDNA sequence is determined. The natural coding sequence of the precursor of the pIgR protein (SEQ ID NO 2) starts with an ATG methionine codon and ends with a TAG stop codon. Within the coding sequence significant features are

a) the presence of a hydrophobic leader sequence immediately following the initiation codon (amino acids 1-18) and
b) a second stretch of hydrophobic amino acids (639-661) corresponding to the transmembrane portion, which separates the extracellular amino-terminal part from the intracellular carboxyterminal portion of pIgR.

The leader peptide is cleaved off from the precursor polypeptide within the cell prior to presentation of the receptor protein on the cell membrane.

Example 2: PCR mutagenesis of human pIgR cDNA

An artificial DNA molecule encoding secretory component is generated by creation of a stop codon immediately before the transmembrane segment using PCR mutagenesis. For this purpose the information contained in DNA sequence encoding pre-pIgR is sufficient, but optionally, vector DNA sequences lying upstream of the ATG initiation codon can be used in the creation of a DNA molecule coding for secretory component.

The polymerase chain reaction (PCR) reaction is done with two synthetic oligonucleotide primers, here termed forward and backward primer (terms relating to the direction of

transcription primed by the respective oligonucleotides relative to the direction of the pIgR translational reading frame).

The forward PCR primer is chosen such that the functional properties of the leader peptide are preserved. The simplest way to achieve this is, is not altering the natural leader peptide sequence. The primer sequence upstream of the ATG codon is further chosen such that a convenient restriction site, which is compatible with a restriction site in a suitable expression vector, is created. One preferred restriction site is that of the restriction endonuclease HindIII, AAGCTT. A preferred DNA molecule for PCR modification is pIgR cDNA cloned in vector pCB6. In this vector the pIgR cDNA is cloned downstream of the major immediate early promoter of the human cytomegalovirus. In this case, a primer hybridizing to the cytomegalovirus promoter segment can be used, preserving restriction sites already present in the vector between the priming site and the position of the ATG codon. A preferred oligonucleotide primer is 5' PCR primer 1 (SEQ ID NO 5).

A second criterion used in the design of the oligonucleotide is that the sequence around the ATG codon is such that the initiation of protein synthesis at the ATG is efficient. The DNA sequence found upstream of the ATG codon in natural mRNAs is not always optimal as under natural conditions only limited amounts of a protein are needed. The criteria for optimal initiation efficiency are known (Kozak *et al.*, Nucleic Acids Res. (1987), **15**, 8125-8148; Peabody D.S. in Setlow J.K. ed. Genetic Engineering Vol. **12**, pp. 99-76). It is preferred that the nucleotide immediately following the ATG is G. This is not the case in the natural pIgR cDNA, but can be achieved be designing the forward PCR primer accordingly. However, this can only be achieved by changing the codon of the second amino acid, leucine, to one starting with G, coding either for valine, alanine, aspartate, glutamate or glycine. The preferred amino acid is valine. This is a conservative substitution as both amino acids belong to the class of hydrophobic amino acids. The functional properties of the peptide leader sequence in promoting secretion of the protein are preserved. One preferred DNA sequence immediately upstream of the ATGG is CC, thus situating the ATG codon within the recognition site of the restriction endonuclease NcoI, CCATGG). This NcoI site is compatible with NcoI sites is many efficient expression vectors. Another preferred DNA sequence immediately upstream of the ATGG is GCCACC. A preferred 5' PCR primer, which creates also a HindIII site upstream of the ATG, is 5' PCR primer 2 (SEQ ID NO 6).

The backward primer is designed with two main criteria in mind. Firstly, it serves to generate a stop codon at position 1906 just upstream of the hydrophobic transmembrane

segment of the plgR protein. The primary translation product will thus terminate with Ser-Glu-Glu-Gln-Gly-Gly-COOH. Secondly, it serves to create downstream of the new stop codon a restriction endonuclease site compatible with a restriction site in a suitable expression vector. One preferred stop codon is TGA and a preferred restriction site is that of Xbal, TCTAGA as created by preferred 3' PCR primer 1 (SEQ ID NO 7).

Example 3: Polymerase chain reaction

A DNA fragment is transcribed by a heat-stable DNA polymerase using a specific forward and 3' PCR primer as described in example 2 and with plgR cDNA cloned in a plasmid vector as template. The PCR reaction is done (according to Sambrook *et al.*, Molecular Cloning: A laboratory manual, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY, 1989) in a buffered solution containing the four deoxyribonucleotide triphosphates and the temperature is of the incubation controlled such that multiple cycles of primer-driven transcription of the SC-coding fragment occur. This produces a DNA fragment terminating with DNA sequences of the two oligonucleotide primers. A HindIII-Xbal fragment encoding SC is generated from this DNA fragment by first separating the SC DNA fragment from the primers and other components of the PCR reaction and then digesting this DNA to completion with HindIII and Xbal. Subsequently, the 1.9 kb HindIII-Xbal fragment purified from the restriction enzyme reaction and is ready for ligation to the restricted expression vector DNA.

The PCR product generated in this way with 5' PCR primer 1 (SEQ ID NO 5) and 3' PCR primer 1 (SEQ ID NO 7) is called SC DNA fragment 1 (SEQ ID NO 3). The PCR product generated in this way with 5' PCR primer 2 (SEQ ID NO 6) and 3' PCR primer 1 (SEQ ID NO 7) is called SC DNA fragment 2 (SEQ ID NO 4).

Example 4: Construction of SC expression vectors with the geneticin resistance gene

The artificial DNA segment encoding secretory component (SC) is inserted between restriction endonuclease sites in a suitable mammalian cell expression vector. One preferred expression vector is pCB6, of which the main features are listed in Table 1 and that can be synthesized easily with standard methods in genetic engineering:

Table 1

Map position (kb)	Origin of DNA segment	Function of DNA segment
0 - 0.75	human cytomegalovirus	immediate early promoter (for cDNA)
0.75 - 0.80	synthetic	multiple restriction sites, including (in order of cleavage position) by HindIII and XbaI
0.8 - 1.44	human growth hormone	polyadenylation signal for cDNA
1.44 - 1.78	SV40	early promoter (for NEO gene)
1.78 - 3.12	transposon Tn5	NEO gene (geneticin resistance)
3.12 - 3.36	SV40	polyadenylation signal for NEO gene
3.36 - 6.20	pTZ18R *(Pharmacia)*	replication in *E. coli*

The vector pCB6 or pCB6 containing the pIgR cDNA (pCB6pIgR) is cut to completion with HindIII and XbaI and the 6.1 kb fragment is isolated. This DNA fragment is ligated using DNA ligase (from bacteriophage T4 or another enzyme with ligation activity) to SC DNA fragment 1. This ligation product is used to transform *E. coli* DH5α and ampicillin resistant strains are isolated. A plasmid DNA is isolated from such an ampicillin resistant strain is termed pCB6-SC and is structured as pCB6, but with the 1.9 kb SC DNA fragment 1 (Example 3) replacing the small HindIII-XbaI fragment of pCB6.

An alternative expression vector is constructed from pCB6-SC, by replacing the major immediate early promoter from the human cytomegalovirus, by the more powerful major immediate early promoter from the mouse cytomegalovirus (mCMV). This promoter is obtained together with the beginning of the ampicillin resistance gene as a 1.1 kb PvuI-HindIII fragment from plasmid pCGA93D-PPREN (Asselbergs *et al.* J. Biotechnol. (1994), **32**, 191-202). In parallel, pCB6 is cut with PvuI and HindIII and the largest fragment (6.1 kb), which contains all the structural elements of the expression vector (see table 1) except of the human cytomegalovirus promoter and the beginning of the pTZ18R-derived ampicillin resistance gene, is purified. The 1.1 kb and the 6.1 kb fragment are ligated and after transformation of *E. coli* with the ligation product ampicillin resistant colonies containing plasmid pMC-SC are obtained. pMC-SC is functionally equivalent to pCB-6 (see table 1) except that the human cytomegalovirus promoter is replaced by the murine one.

Example 5 : Construction of SC expression plasmid pCGA93D-SC

The artificial DNA segment encoding secretory component (SC) is inserted between restriction endonuclease sites in a suitable mammalian cell expression vector. One preferred expression vector is pCGA93D-PPREN which is constructed according to Asselbergs *et al.*, J. Biotech. (1994), **32**, 191-202, and of which the main features are listed in Table 2:

Table2

Map position (kb)	Origin of DNA segment	Function of DNA segment
0 - 0.5	mouse cytomegalovirus	immediate early promoter (for cDNA)
0.5 - 1.9	synthetic linkers flanking human prorenin cDNA	The prorenin cDNA is preceded by a HindIII site and followed by an XbaI and a BamHI site.
1.9 -3.1	rabbit beta-globin	splicing and polyadenylation signals for cDNA
3.1 - 3.5	human adenovirus type 2	promoter + splicing donor signals (for DHFR gene)
3.5 - 3.7	mouse immunoglobulin	splicing acceptor signals (for DHFR gene)
3.7 - 4.3	mouse DHFR cDNA	dihydrofolate reductase coding DNA
4.3 - 4.7	SV40	polyadenylation signal for DHFR gene
4.7 - 7.9	pBRd	replication in *E. coli*. Bacterial selection genes for tetracycline and ampicillin resistance. There is second BamHI restriction site at 5.0 kb and a SalI restriction site at 5.2 kb, both in the tetracyclin resistance gene.

The vector pCGA93D-PPREN is cut to completion with BamHI and the large 4.9 kb fragment is isolated, self-ligated and used to transform *E. coli* DH5α (the plasmid is termed pINTERMED1). From an ampicillin resistant strain plasmid pINTERMED1 (4.9 kb) is

purified. In pINTERMED1 the HindIII and XbaI sites flanking the prorenin cDNA are unique restriction sites. pINTERMED1 is cut to completion with HindIII and XbaI and the 3.5 kb fragment is isolated. This DNA fragment is ligated using DNA ligase (from bacteriophage T4 or another enzyme with ligation activity) to SC DNA fragment 2 (Example 3). This ligation product is used to transform *E. coli* DH5α and ampicillin resistant strains are isolated. A plasmid DNA is isolated from such an ampicillin resistant strain is termed pINTERMED2 (6.8 kb). pINTERMED2 is cut to completion with SalI and XbaI and the large fragment (6.5 kb) is isolated. In parallel pCGA93D-PPREN is cut to completion with SalI and XbaI and the 3.3 kb fragment is isolated. The two DNA fragments are ligated and the product is used to transform *E. coli* DH5α. From an ampicillin and tetracyclin resistant strain thus obtained pCGA93D-SC is isolated. This plasmid is structured like pCGA93D-PPREN, but with the SC coding DNA replacing the preprorenin coding DNA. In pCGA93D-SC the SC coding DNA starts with an NcoI site and the DNA sequence around the ATG initiation codon of SC allows efficient initiation of translation of the recombinant SC mRNA.

Example 6 : Expression of human secretory component in CHO SSF3 cell transfected with pCB6-SC or pMC-SC

CHO SSF 3 cells are known (Gandor C.R., Establishment and characterization of growth-factor-prototrophic Chinese hamster ovary (CHO) cell lines for the production of recombinant proteins, Zürich: Dissertation #10087, Swiss Federal Institute of Technology, 1993). A cell stock is maintained in FMX-8 medium (Dr. F. Messi Cell Culture Technologies, Rohrstrasse 29, CH-8152 Glattbrugg/Zürich) without further additives. If transfection is going to be performed without serum, one to ten million cells are pelleted at low speed in a centrifuge and the cells are resuspended at 200'000 cells/ml in fresh FMX-8 medium of 37ºC. In the alternative procedure, a dense culture of CHO SSF 3 cells is diluted 10-fold in FMX-8 medium with 4% fetal calf serum and plated in 3 cm diameter tissue culture Petri dishes. Cultured in this way the CHO SSF 3 cells form a cell monolayer, which adheres to the plastic of the Petri dish. When this monolayer is 30-50% confluent, they can be used for transfection.

The solutions for transfection are prepared in a polystyrene vessels to prevent adsorption of the cationic lipid used and the complex formed of the cationic lipid and DNA to the vessel wall. The pCB6-SC (example 3) DNA is prepared for transfection as follows: 4 µg plasmid DNA is dissolved in 0.1 ml FMX-8 medium. Separately 14 µl cationic lipid solution (lipofectin, GIBCO) is diluted in 0.1 ml FMX-8 medium. Subsequently, the DNA solution and

the lipofectin solution are carefully mixed and incubated at room temperature for 15 min to allow formation of a lipofectin-DNA complex.

For the serum-free procedure, the lipofectin-DNA complex is mixed with the CHO SSF 3 cell suspension (end volume 0.5 ml) and placed in a CO_2 incubator (5% CO_2) at 37°C. After a 16h 1 ml FMX-8 is added to the cells and the incubation continued. After another 24h the cells are diluted 10-fold in fresh FMX-8 + 0.25 mg/ml geneticin, divided in 96-well microtiter plate and incubated at 37°C until after approximately 3 weeks incubation dense cell growth is detected in some of the wells. An aliquot of the medium from such wells is assayed for the presence of human SC. Cells from the wells in which SC is detected are transferred to larger culture vessels and expanded until sufficient cells are obtained to inoculate a stirred tank bioreactor.

In the alternative procedure the serum-containing medium is suctioned off, cells are rinsed with FMX-8 without serum and 0.3 ml FMX-8 medium without serum is added. To this medium the lipofectin-DNA complex solution is added and the cells are incubated for 5 h in a CO_2 incubator (5% CO_2) at 37°C, after which 1 ml of FMX-8 with 4% serum is added. 24h later the cells are trypsinized with undiluted 0.25 % porcine trypsin (JRH Biosciences Lenexa, Kansas U.S.A) as described in Asselbergs *et al.*, J. Biotechnol. (1992). **26**, 301-313, diluted 20-fold in FMX-8 with 4% serum and 1 mg/ml geneticin and plated in Petri dishes. After 2-3 weeks colonies of geneticin-resistant cells have developed, which are individually scraped off and transferred to a 24-well microtiter plate. FMX-8 without serum with 0.25 mg/ml geneticin is added to the cells. After a week a dense culture of mostly non-adherent cells has developed. The concentration of SC in the conditioned medium from each well is measured by ELISA (as described in example 10) and cells from the wells in which a high amount SC is detected are transferred to larger culture vessels and expanded until sufficient cells are obtained to inoculate a stirred tank bioreactor.

One cell line CHO SSF 3 producing human SC is designated SSF3-HSC-1 and is used for production of human SC in stirred tank bioreactor. The cell population produces more than 10 µg SC per million cells day.

Using the same procedures cells, which are transfected with pMC-SC instead of pCB6-SC, are obtained. One pMC-SC -transfected CHO SSF3 cell line producing human SC is designated CHO-SSF3/pMC-SC3 (DSM ACC2203) and is used for production of human SC

in a stirred bioreactor. The cell population produces more than 10 mg SC per million cells per day.

Example 7: Expression of human secretory component in CHO SSF 3 cells transfected with pCGA93D-SC

CHO SSF 3 cells and lipofectin-DNA complex are prepared as described above. (Example 6), except that instead of plasmid pCB6-SC plasmid pCGA993D-SC (Example 5) is used.

For the serum-free procedure, the lipofectin-DNA complex is mixed with the CHO SSF 3 cell suspension (end volume 0.5 ml) and placed in a CO_2 incubator (5% CO_2) at 37°C. After a 16h 1 ml FMX-8-minus (FMX-8 lacking glycine, hypoxanthine and thymidine) is added to the cells and the incubation continued. After another 24h the cells are diluted 10-fold in fresh FMX-8-minus + 5 nM methotrexate, divided in 96-well microtiter plate and incubated at 37°C until after approximately 3 weeks incubation dense cell growth is detected in some of the wells. An aliquot of the medium from such wells is assayed for the presence of human SC. Cells from the wells in which SC is detected are transferred to larger culture vessels and expanded until sufficient cells are obtained for selection of cell lines with increased methotrexate resistance (Example 8) or to inoculate a stirred tank bioreactor.

In the alternative procedure the serum-containing medium is suctioned off, the cells are rinsed with FMX-8-medium without serum and 0.3 ml FMX-8-medium without serum is added. To this medium the lipofectin-DNA complex solution is added and the cells are incubated for 5 h in a CO_2 incubator (5% CO_2) at 37°C, after which 1 ml of FMX-8-minus with 4% dialyzed serum is added. 24h later the cells are trypsinized, diluted 20-fold in FMX-8-minus with 4% dialyzed serum and 5 nM methotrexate and plated in Petri dishes. After 2-3 weeks colonies of methotrexate-resistant cells have developed, which are individually scraped off and transferred to a 24-well microtiter plate. FMX-8-minus without serum with 5 nM methotrexate is added to the cells. After a week a dense culture of mostly non-adherent cells has developed. The concentration of SC in the conditioned medium from each well is measured and cells from the wells in which a high amount SC is detected are transferred to larger culture vessels and expanded until sufficient cells are obtained for selection of cell lines with increased methotrexate resistance (Example 8) or to inoculate a stirred tank bioreactor.

One CHO SSF 3 cell line producing human SC is designated SSF3-HSC-M1 and is used for production of human SC in stirred tank bioreactor. The cell population produces more than 10 µg SC per million cells day.

Example 8: Methotrexate selection of cell lines with increased number of copies of pCGA93D-SC

It is possible to select for spontaneous amplification of the plasmid DNA integrated into the chromosome of the transfected CHO SSF 3 cells. To achieve this, the transfected CHO SSF 3 cells selected to be resistant to 5 nM methotrexate are subcultured in gradually increasing concentrations of methotrexate. Like the transfection, this procedure can be done in protein-free medium or in medium containing serum. The latter method has the advantage that it is easier to isolate individual colonies of cells resistant to the higher methotrexate concentration, but cells have to readapted to growth in protein-free medium. This is done by gradually diluting out the serum over a two week growth period.

For the serum-free procedure, the cells cultured in FMX-8-minus (FMX-8 lacking glycine, hypoxanthine and thymidine) plus 5 nM methotrexate are diluted to a cell density of approximately 500 cells/ml in medium with the new methotrexate concentration and divided over several 96-well microtiter culture plates. It is known that the frequency of amplification is approximately one in 10000 and that small increments of the methotrexate concentration favor the development of resistance due to gene amplification rather than other gene alterations (Kaufman R.J., Methods in Enzymology (1990), **185**, 537-566). Therefore, the selection is initiated at about twice the initial concentration of 5 nM methotrexate. In about one in 20 wells a dense culture of cells with increased methotrexate resistance develops over a period of 2-3 weeks. Such cells are transferred to larger culture vessels and expanded until sufficient cells are obtained to test the specific production of secretory component. Cells with higher specific SC production are obtained with a frequency of 20-40% of the more resistant subcultures. This selection procedure is repeated several times, each time raising the methotrexate concentration 1.5-2.5-fold. When a concentration of 50-150 nM methotrexate is reached a cell population producing more than 10 µg SC per million cells day is obtained. These cells are then transferred to an appropriate bioreactor for large scale SC production

In the alternative procedure, the cells are cultured adherent to plastic in FMX-8-minus medium with 4% dialyzed fetal calf serum initially with 5 nM methotrexate. The cells are

trypsinized and replated at 20000 cells/ml in several 10 cm diameter Petri dishes (10 ml medium total) in the same medium but with the higher concentration of methotrexate. Like stated above, the selection is initiated at about twice the initial concentration of 5 nM methotrexate. After about 3 weeks colonies of more resistant cells are developed which are individually scaped off and transferred to multiwell dishes with medium with the same methotrexate concentration. When sufficient cells are obtained the specific production of SC is measured. Cells with higher specific SC production are obtained with a frequency of 20-40% of the more resistant subcultures. This selection procedure is repeated several times, each time raising the methotrexate concentration 1.5-2.5-fold. When a concentration of 50-150 nM methotrexate is reached a cell population producing more than 10 µg SC per million cells day is obtained. The cell line thus obtained is readapted to growth in protein-free FMX-8 minus by gradually over a period of 1-2 weeks lowering the serum concentration. These cells are then transferred to an appropriate bioreactor for large scale SC production.

Example 9: ELISA assay for human SC in conditioned medium of transfected CHO SSF 3 cells

Polystyrene microtiter plates are coated overnight at 4°C with 100 µl/well of 10 µg/ml rabbit anti-human SC immunoglobulin (DAKO Code nr. A187) dissolved in PBS without $MgCl_2$ and $CaCl_2$ (GIBCO) containing 0.1 mg/ml of the bacteriostatic sodium ethyl mercurithiosalicylate. Subsequently, the plates are rinsed three times with in washing solution: PBS lacking $MgCl_2$ and $CaCl_2$ containing 0.05% Tween-20 (EIA-grade, BIORAD) and 0.1 mg/ml sodium ethyl mercurithiosalicylate. Non-specific protein binding sites on the polystyrene are neutralized by incubation with blocking buffer: PBS containing 2% bovine serum albumin (BSA), 0.5% rabbit serum and 0.05% Tween-20. Subsequently, the plates are rinsed three times with washing solution. A 100 µl sample of an SC-containing solution (conditioned medium, column fraction from a purfication etc.) diluted in blocking solution is added to each well and incubated overnight at 4°C. Subsequently, the plates are rinsed three times with washing solution. Next, 50 µl of a solution containing 250 ng/ml biotinylated anti-human SC immunoglobulin in blocking buffer is added followed by an incubation of 1 h at room temperature. This biotinylated antibody is prepared by treatment of rabbit anti-human SC immunoglobulin (DAKO Code nr. A187) with aminohexanoyl-biotin-n-hydroxysuccinimide ester (Zymed) according to the instructions of the manufacturer. Subsequently, the plates are rinsed three times with washing solution. Next, 50 µl of avidin crosslinked to horseradish peroxidase (Zymed) 1000-fold diluted in blocking buffer is added followed by an

incubation of 1 h at room temperature. After washing the plate three times with washing solution, 150 µl/well of enzyme color substrate solution, 21 mg/ml citric acid and 35.6 mg/ml Na_2HPO_4 at pH 4.5 containing 1.5 mg/ml O-phenyldiamine (SIGMA) and 1 µl/ml 30% H_2O_2, is added. After 15 min. at 37°C the enzyme reaction is stopped by addition of 50 µl/well of 0.5 N H_2SO_4 and the absorption measured at 492 nm. Relative concentration (titer) of SC is calculated as an arbitrary unit (AU) i.e. the absorbance measured multiplied by the volume and dilution factor of the sample. Alternatively, the assay is standardized by testing a dilution series of purified recombinant human SC in parallel.

Example 10: ELISA assay for binding of SC to human IgA

Polystyrene microtiter plates are coated overnight at 4°C with 100 µl/well of either 10 µg/ml human serum albumin (negative control, Sigma A-6003), human IgG (Sigma I-4506), IgM (Sigma I-8640), IgA (Sigma, I-0633) or rabbit anti-human SC immunoglobulin (DAKO Code nr. A187) dissolved in PBS without $MgCl_2$ and $CaCl_2$ (GIBCO) containing 0.1 mg/ml of the bacteriostatic sodium ethyl mercurithiosalicylate. Subsequently, the plates are rinsed three times with in washing solution : PBS lacking $MgCl_2$ and $CaCl_2$ containing 0.05% Tween-20 (EIA-grade, BIORAD) and 0.1 mg/ml sodium ethyl mercurithiosalicylate. Non-specific protein binding sites on the polystyrene are neutralized by incubation with blocking buffer: PBS containing 2% bovine serum albumin (BSA), 0.5% rabbit serum and 0.05% Tween-20. Subsequently, the plates are rinsed three times with washing solution. A 100 µl sample of an SC-containing solution diluted in blocking solution is added to each well and incubated overnight at 4°C. Subsequently, the plates are rinsed three times with washing solution. Next, 50 µl of a solution containing 250 ng/ml biotinylated anti-human SC immunoglobulin in blocking buffer is added followed by an incubation of 1 h at room temperature. This biotinylated antibody is prepared by treatment of rabbit anti-human SC immunoglobulin (DAKO Code nr. A187) with aminohexanoyl-biotin-n-hydroxysuccinimide ester (Zymed) according to the instructions of the manufacturer. Subsequently, the plates are rinsed three times with washing solution. Next, 50 µl of avidin crosslinked to horseradish peroxidase (Zymed) 1000-fold diluted in blocking buffer is added followed by an incubation of 1 h at room temperature. After washing the plate three times with washing solution, 150 µl/well of enzyme color substrate solution, 21 mg/ml citric acid and 35.6 mg/ml Na_2HPO_4 at pH 4.5 containing 15 mg/ml O-phenyldiamine (SIGMA) and 1 µl/ml 30% H_2O_2, is added. After 15 min. at 37°C the enzyme reaction is stopped by addition of 50 µl/well of 0.5 N H_2SO_4 and the absorption measured at 492 nm. Relative concentration (titer) of SC is calculated as an arbitrary unit (AU) i.e. the absorbance measured multiplied by the volume and dilution factor

of the sample. Alternatively, the assay is standardized by testing a dilution series of purified recombinant human SC in parallel.

Example 11: Production of SC of IgA in small pilot scale suspension culture in serum and protein free cultivated CHO SSF 3 cells.

All cell cultivations are performed as suspended repeated step-wise-fed-batch cultivations in 10 L glass-bioreactors with marine type impellers. After having reached maximal cell density and working volume, indicated by beginning stationary growth phase, 90% of the cell suspension is harvested and the remaining 10% are diluted by a factor 1:10 with fresh medium. The process is controlled by on-line control loops for temperature, pH and pO_2. Cell concentration, cell viability and product concentration (end concentration) are off-line determined. The basal medium consists of FMX-8 (Dr. F. Messi AG, Zürich, Switzerland). The cells however are proved to be shear sensitive with respect to their production kinetics. The growth kinetics, however, are only slightly affected (see below). Medium is therefore supplied with Pluronic F-68 (P-1300, SIGMA) as productivity enhancer. The two different medium configurations are compared below.

Example 11.1: Batch cultivation with FMX-8 medium

After inoculation of the bioreactor with 1.2×10^5 cells/ml and 2.5 L working volume the cells are grown up to 9.8×10^5 cells/ml. During growth phase the working volume is increased twice by the addition of 3 L of fresh medium after 4 d and 2 L of fresh medium after 7 d respectively. This batch cultivation results in a final working volume of 8 L after 9 d of culture and with a final concentration of 109 mg/L of SC of IgA. The mean doubling time of the cells is 2.82 d and the daily yield of the SC of IgA is 12.1 mg/L/d.

Example 11.2: Batch cultivation with FMX-8 medium, supplied with Pluronic F-68

The FMX-8 medium is supplied with 0.05% (w/v) Pluronic F-68 as productivity enhancer. After inoculation of the bioreactor with 2.3×10^5 cells/ml (from Example 8)and 2.5 L working volume the cells are grown up to 1.1×10^6 cells/ml. During growth phase the working volume is increased 3 times by the addition of 2.5 L of fresh medium after 1 d, 3 L after 3 d and 1 L after 4 d respectively. This batch cultivation results in a final working volume of 9 L after 7 d of culture and with a final cell concentration of 1.1×10^6 cells/ml and a final concentration of 229 mg/L of SC of IgA. With 2.26 d the mean doubling time is only 20% lower than without Pluronic supplementation. On the contrary, the daily yield of the SC of IgA is with 32.7 mg/L/d 3 times higher than without Pluronic supplementation.

Example 12: Human secretory component produced by Chinese hamster ovary cells in the absence and presence of Pluronic.

After removal of the residual cellular biomass human secretory component (hSC) is isolated from the FMX-8-based cell culture medium. Protease inhibitors, like PMSF (0.1 M in 2-propanol) and E-64 (7 mM in 50% (v/v) aqueous ethanol), and NaCl are added to give final concentrations of 1 mM, 2.8 µM and 0.5 M, respectively, and the pH is adjusted to 5.6 with 4 N HCl. After addition of $CaCl_2$ and $MnCl_2$ to give a final concentration of 1 mM each, 150 ml Concanavalin A Sepharose (Pharmacia) slurry are added and suspended overnight at 4°C. The immobilized lectin is collected above a glassfilter, washed with 5 mM sodiumacetate pH 5.6, containing 0.5 M NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$ (binding buffer), and packed into a 2.6 cm x 30 cm column-housing. Concanavalin A-bound protein is eluted with binding buffer, containing 0.5 M methyl-α-D-mannopyranoside. The hSC-containing fraction are concentrated and dialyzed against 50 mM sodiumacetate pH 5.5, containing 0.15 M NaCl and 0.02 (w/v) NaN_3, by ultrafiltration using an YM10® membrane (AMICON) in an Amicon cell. Further fractionated is achieved by gel-permeation chromatography on a column (1.6 cm x 61 cm) of Sephacryl S-300 (Pharmacia). The column is eluted with 50 mM sodiumacetate pH 5.5, containing 0.15 M NaCl, and the effluent monitored at 278 nm. The major peak, containing hSC as monitored by SDS/PAGE, is collected and concentrated by ultrafiltration using an YM10 membrane in an Amicon cell to a concentration of about 20 mg/ml. Approximately 35 mg of hSC are isolated per liter of cell culture medium as measured using the Bio-Rad protein assay using IgG as a standard. The final preparation shows an apparent purity in excess of 95%, and is stored at 4ºC in the presence of 0.02% (w/v) NaN_3.

Example 13: Crystallization of recombinant soluble polymeric Ig receptor (hSC)

The crystallization experiments are performed using the hanging drop method.

2 µl of the SC solution from example 12 at a concentration of about 5 mg/ml in 10 mM sodium acetate buffer at pH 5.5 containing 100 mM NaCl is mixed with an equal volume of precipitating agent buffer. The precipitating agent buffer can be :

A) 0.1 M Na-citrate, 1.0 M ammonium phosphate and 0.02% NaN_3; or
B) 0.1 M HEPES, 1.5 M $(Li)_2SO_4$ and 0.02% NaN_3

The protein and precipitating agent solutions are mixed on a glass cover slide. 1000 µl of the respective precipitating buffer solution (reservoir) is placed into wells of Linbro plates. The glass cover slide with the hanging drop of protein solution is placed over the well. Equilibration of the concentration of precipitating agent between reservoir and hanging drops *via* the vapor phase occurs within a few weeks at room temperature. During this period plate-like crystals of hSC appear in the drops.

Example 14: Sialic acid analysis of recombinant soluble polymeric Ig receptor (hSC)

The analysis of sialic acids is carried out essentially as described in Harra *et al.*, Anal. Biochem. (1989), **179**, 162-166. An aliquot of 236 µg recombinant soluble polymeric Ig receptor (hSC) in 10 µl 0.05 M sodiumacetate pH 5.5, containing 0.15 M sodiumchloride is dried under reduced pressure and solved in 200 µl 2 M acetic acid, heated for 3 h at 80°C. Released sialic acids are converted into fluorescent derivatives by the addition of 200 µl 7.0 mM 1,2-diamino-4,5-methylenedioxybenzene (DMB, Sigma) in 1.4 M acetic acid, containing 0.75 M β-mercaptoethanol and 18 mM sodium dithionate at 50°C for 2.5 h. N-acetylneuraminic acid (Neu5Ac, Sigma), N-glycolylneuraminic acid (Neu5Gc, Sigma), human serumtransferrin (hST, Serva), bovine glycoprotein fraction VI (bGP, Miles Laboratories) and a mixture of Neu5Ac, Neu5Gc, N-acetyl-7-O-acetylneuraminic acid ($Neu5,7Ac_2$), N-glycolyl-9-O-acetylneuraminic acid ($Neu9Ac_5Gc$), N-acetyl-9-O-acetyl-neuraminic acid ($Neu5,9Ac_2$), N-acetyl-7(8),9-di-O-acetylneuraminic acid ($Neu5,7(8),9Ac_3$) (Oxford Glycosystems) are taken through the procedure as standards. HPLC analysis is carried out on a Waters Novapak C^{18} 4-µm (60 Å) column (3.9 x 150 mm) fitted into a Waters 840 chromatography system equipped with two model 510 HPLC pumps, a WISP model 712 sample processor, a model 490 programmable multi-wavelength detector and a Kratos GM 970 fluorescence detector operating at an excitation wavelength of 373 nm, detecting emission at wavelength >418 nm using a cut-off filter. Simultaneously, the absorbance of the eluent is monitored at a wavelength of 373 nm. Elutions are performed isocratically using acetonitrile : methanol : water (6.4 : 4.9 : 88.7, v/v/v) as eluent at a flow rate of 0.7 ml/min.

The HPLC profiles of the DMB sialic acids derived from hST and bGP show only Neu5Ac for hST (as described in Spik *et al.*, FEBS Lett. (1975), **50**, 296-299 and Hokke *et al.*, FEBS Lett. (1990), **275**, 9-14) and a mixture of Neu5Gc and Neu5Ac in a ratio of 1.0 : 1.0 for bGP. The HPLC pattern of the DMB sialic acids derived from hSC show peaks at the elution

positions of Neu5Gc and Neu5Ac, respectively. The content of Neu5Ac in hSC is more than 99.9% and that of Neu5Gc less than 0.1%.

DEPOSITIONS

The following microorganism strains were deposited at the Deutsche Sammlung von Mikroorganismen (DSM), Mascheroder Weg 1b, D-38124 Braunschweig (accession numbers and deposition dates given):

CHO SSF 3 / pMC-SC3	DSM ACC2203	deposited on Dec. 15, 1994

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: CIBA-GEIGY AG

(B) STREET: Klybeckstr. 141

(C) CITY: Basel

(E) COUNTRY: Switzerland

(F) POSTAL CODE (ZIP): 4002

(G) TELEPHONE: +41 61 69 11 11

(H) TELEFAX: + 41 61 696 79 76

(I) TELEX: 962 991

(ii) TITLE OF INVENTION: Production of Recombinant Secretory Component

(iii) NUMBER OF SEQUENCES: 7

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2405 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:111..2402

(D) OTHER INFORMATION:/product= "original pIgR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
CGGTAGGCGT GTACGGTGGG AGGTCTATAT AGCAGAGCTC GTTTAGTGAA CCGTCAGAAT      60

TAATTCAGAT CTGGTACCAC GCGTATCGAT AAGCTTGAAT TCCACCAGCA ATG CTG         116
                                                          Met Leu
                                                            1

CTC TTC GTG CTC ACC TGC CTG CTG GCG GTC TTC CCA GCC ATC TCC ACG         164
Leu Phe Val Leu Thr Cys Leu Leu Ala Val Phe Pro Ala Ile Ser Thr
          5                  10                  15

AAG AGT CCC ATA TTT GGT CCC GAG GAG GTG AAT AGT GTG GAA GGT AAC         212
Lys Ser Pro Ile Phe Gly Pro Glu Glu Val Asn Ser Val Glu Gly Asn
     20                  25                  30

TCA GTG TCC ATC ACG TGC TAC TAC CCA CCC ACC TCT GTC AAC CGG CAC         260
Ser Val Ser Ile Thr Cys Tyr Tyr Pro Pro Thr Ser Val Asn Arg His
 35                  40                  45                  50

ACC CGG AAG TAC TGG TGC CGG CAG GGA GCT AGA GGT GGC TGC ATA ACC         308
Thr Arg Lys Tyr Trp Cys Arg Gln Gly Ala Arg Gly Gly Cys Ile Thr
                 55                  60                  65

CTC ATC TCC TCG GAG GGC TAC GTC TCC AGC AAA TAT GCA GGC AGG GCT         356
Leu Ile Ser Ser Glu Gly Tyr Val Ser Ser Lys Tyr Ala Gly Arg Ala
             70                  75                  80

AAC CTC ACC AAC TTC CCG GAG AAC GGC ACA TTC GTG GTG AAC ATT GCC         404
Asn Leu Thr Asn Phe Pro Glu Asn Gly Thr Phe Val Val Asn Ile Ala
         85                  90                  95
```

```
CAG CTG AGC CAG GAT GAC TCC GGG CGC TAC AAG TGT GGC CTG GGC ATC      452
Gln Leu Ser Gln Asp Asp Ser Gly Arg Tyr Lys Cys Gly Leu Gly Ile
    100                 105                 110

AAT AGC CGA GGC CTG TCC TTT GAT GTC AGC CTG GAG GTC AGC CAG GGT      500
Asn Ser Arg Gly Leu Ser Phe Asp Val Ser Leu Glu Val Ser Gln Gly
115                 120                 125                 130

CCT GGG CTC CTA AAT GAC ACT AAA GTC TAC ACA GTG GAC CTG GGC AGA      548
Pro Gly Leu Leu Asn Asp Thr Lys Val Tyr Thr Val Asp Leu Gly Arg
                135                 140                 145

ACG GTG ACC ATC AAC TGC CCT TTC AAG ACT GAG AAT GCT CAA AAG AGG      596
Thr Val Thr Ile Asn Cys Pro Phe Lys Thr Glu Asn Ala Gln Lys Arg
            150                 155                 160

AAG TCC TTG TAC AAG CAG ATA GGC CTG TAC CCT GTG CTG GTC ATC GAC      644
Lys Ser Leu Tyr Lys Gln Ile Gly Leu Tyr Pro Val Leu Val Ile Asp
        165                 170                 175

TCC AGT GGT TAT GTG AAT CCC AAC TAT ACA GGA AGA ATA CGC CTT GAT      692
Ser Ser Gly Tyr Val Asn Pro Asn Tyr Thr Gly Arg Ile Arg Leu Asp
    180                 185                 190

ATT CAG GGT ACT GGC CAG TTA CTG TTC AGC GTT GTC ATC AAC CAA CTC      740
Ile Gln Gly Thr Gly Gln Leu Leu Phe Ser Val Val Ile Asn Gln Leu
195                 200                 205                 210

AGG CTC AGC GAT GCT GGG CAG TAT CTC TGC CAG GCT GGG GAT GAT TCC      788
Arg Leu Ser Asp Ala Gly Gln Tyr Leu Cys Gln Ala Gly Asp Asp Ser
                215                 220                 225

AAT AGT AAT AAG AAG AAT GCT GAC CTC CAA GTG CTA AAG CCC GAG CCC      836
Asn Ser Asn Lys Lys Asn Ala Asp Leu Gln Val Leu Lys Pro Glu Pro
            230                 235                 240
```

```
GAG CTG GTT TAT GAA GAC CTG AGG GGC TCA GTG ACC TTC CAC TGT GCC      884
Glu Leu Val Tyr Glu Asp Leu Arg Gly Ser Val Thr Phe His Cys Ala
        245                 250                 255

CTG GGC CCT GAG GTG GCA AAC GTG GCC AAA TTT CTG TGC CGA CAG AGC      932
Leu Gly Pro Glu Val Ala Asn Val Ala Lys Phe Leu Cys Arg Gln Ser
    260                 265                 270

AGT GGG GAA AAC TGT GAC GTG GTC GTC AAC ACC CTG GGG AAG AGG GCC      980
Ser Gly Glu Asn Cys Asp Val Val Val Asn Thr Leu Gly Lys Arg Ala
275                 280                 285                 290

CCA GCC TTT GAG GGC AGG ATC CTG CTC AAC CCC CAG GAC AAG GAT GGC     1028
Pro Ala Phe Glu Gly Arg Ile Leu Leu Asn Pro Gln Asp Lys Asp Gly
                295                 300                 305

TCA TTC AGT GTG GTG ATC ACA GGC CTG AGG AAG GAG GAT GCA GGG CGC     1076
Ser Phe Ser Val Val Ile Thr Gly Leu Arg Lys Glu Asp Ala Gly Arg
            310                 315                 320

TAC CTG TGT GGA GCC CAT TCG GAT GGT CAG CTG CAG GAA GGC TCG CCT     1124
Tyr Leu Cys Gly Ala His Ser Asp Gly Gln Leu Gln Glu Gly Ser Pro
        325                 330                 335

ATC CAG GCC TGG CAA CTC TTC GTC AAT GAG GAG TCC ACG ATT CCC CGC     1172
Ile Gln Ala Trp Gln Leu Phe Val Asn Glu Glu Ser Thr Ile Pro Arg
    340                 345                 350

AGC CCC ACT GTG GTG AAG GGG GTG GCA GGA AGC TCT GTG GCC GTG CTC     1220
Ser Pro Thr Val Val Lys Gly Val Ala Gly Ser Ser Val Ala Val Leu
355                 360                 365                 370

TGC CCC TAC AAC CGT AAG GAA AGC AAA AGC ATC AAG TAC TGG TGT CTC     1268
Cys Pro Tyr Asn Arg Lys Glu Ser Lys Ser Ile Lys Tyr Trp Cys Leu
                375                 380                 385
```

```
TGG GAA GGG GCC CAG AAT GGC CGC TGC CCC CTG CTG GTG GAC AGC GAG      1316
Trp Glu Gly Ala Gln Asn Gly Arg Cys Pro Leu Leu Val Asp Ser Glu
            390                 395                 400

GGG TGG GTT AAG GCC CAG TAC GAG GGC CGC CTC TCC CTG CTG GAG GAG      1364
Gly Trp Val Lys Ala Gln Tyr Glu Gly Arg Leu Ser Leu Leu Glu Glu
        405                 410                 415

CCA GGC AAC GGC ACC TTC ACT GTC ATC CTC AAC CAG CTC ACC AGC CGG      1412
Pro Gly Asn Gly Thr Phe Thr Val Ile Leu Asn Gln Leu Thr Ser Arg
    420                 425                 430

GAC GCC GGC TTC TAC TGG TGT CTG ACC AAC GGC GAT ACT CTC TGG AGG      1460
Asp Ala Gly Phe Tyr Trp Cys Leu Thr Asn Gly Asp Thr Leu Trp Arg
435                 440                 445                 450

ACC ACC GTG GAG ATC AAG ATT ATC GAA GGA GAA CCA AAC CTC AAG GTA      1508
Thr Thr Val Glu Ile Lys Ile Ile Glu Gly Glu Pro Asn Leu Lys Val
                455                 460                 465

CCA GGG AAT GTC ACG GCT GTG CTG GGA GAG ACT CTC AAG GTC CCC TGT      1556
Pro Gly Asn Val Thr Ala Val Leu Gly Glu Thr Leu Lys Val Pro Cys
            470                 475                 480

CAC TTT CCA TGC AAA TTC TCC TCG TAC GAG AAA TAC TGG TGC AAG TGG      1604
His Phe Pro Cys Lys Phe Ser Ser Tyr Glu Lys Tyr Trp Cys Lys Trp
        485                 490                 495

AAT AAC ACG GGC TGC CAG GCC CTG CCC AGC CAA GAC GAA GGC CCC AGC      1652
Asn Asn Thr Gly Cys Gln Ala Leu Pro Ser Gln Asp Glu Gly Pro Ser
    500                 505                 510

AAG GCC TTC GTG AAC TGT GAC GAG AAC AGC CGG CTT GTC TCC CTG ACC      1700
Lys Ala Phe Val Asn Cys Asp Glu Asn Ser Arg Leu Val Ser Leu Thr
515                 520                 525                 530
```

```
CTG AAC CTG GTG ACC AGG GCT GAT GAG GGC TGG TAC TGG TGT GGA GTG      1748
Leu Asn Leu Val Thr Arg Ala Asp Glu Gly Trp Tyr Trp Cys Gly Val
                535                 540                 545

AAG CAG GGC CAC TTC TAT GGA GAG ACT GCA GCC GTC TAT GTG GCA GTT      1796
Lys Gln Gly His Phe Tyr Gly Glu Thr Ala Ala Val Tyr Val Ala Val
            550                 555                 560

GAA GAG AGG AAG GCA GCG GGG TCC CGC GAT GTC AGC CTA GCG AAG GCA      1844
Glu Glu Arg Lys Ala Ala Gly Ser Arg Asp Val Ser Leu Ala Lys Ala
        565                 570                 575

GAC GCT GCT CCT GAT GAG AAG GTG CTA GAC TCT GGT TTT CGG GAG ATT      1892
Asp Ala Ala Pro Asp Glu Lys Val Leu Asp Ser Gly Phe Arg Glu Ile
    580                 585                 590

GAG AAC AAA GCC ATT CAG GAT CCC AGG CTT TTT GCA GAG GAA AAG GCG      1940
Glu Asn Lys Ala Ile Gln Asp Pro Arg Leu Phe Ala Glu Glu Lys Ala
595                 600                 605                 610

GTG GCA GAT ACA AGA GAT CAA GCC GAT GGG AGC AGA GCA TCT GTG GAT      1988
Val Ala Asp Thr Arg Asp Gln Ala Asp Gly Ser Arg Ala Ser Val Asp
                615                 620                 625

TCC GGC AGC TCT GAG GAA CAA GGT GGA AGC TCC AGA GCG CTG GTC TCC      2036
Ser Gly Ser Ser Glu Glu Gln Gly Gly Ser Ser Arg Ala Leu Val Ser
            630                 635                 640

ACC CTG GTG CCC CTG GGC CTG GTG CTG GCA GTG GGA GCC GTG GCT GTG      2084
Thr Leu Val Pro Leu Gly Leu Val Leu Ala Val Gly Ala Val Ala Val
        645                 650                 655

GGG GTG GCC AGA GCC CGG CAC AGG AAG AAC GTC GAC CGA GTT TCA ATC      2132
Gly Val Ala Arg Ala Arg His Arg Lys Asn Val Asp Arg Val Ser Ile
    660                 665                 670
```

```
AGA AGC TAC AGG ACA GAC ATT AGC ATG TCA GAC TTC GAG AAC TCC AGG      2180
Arg Ser Tyr Arg Thr Asp Ile Ser Met Ser Asp Phe Glu Asn Ser Arg
675                 680                 685                 690

GAA TTT GGA GCC AAT GAC AAC ATG GGA GCC TCT TCG ATC ACT CAG GAG      2228
Glu Phe Gly Ala Asn Asp Asn Met Gly Ala Ser Ser Ile Thr Gln Glu
                695                 700                 705

ACA TCC CTC GGA GGA AAA GAA GAG TTT GTT GCC ACC ACT GAG AGC ACC      2276
Thr Ser Leu Gly Gly Lys Glu Glu Phe Val Ala Thr Thr Glu Ser Thr
            710                 715                 720

ACA GAG ACC AAA GAA CCC AAG AAG GCA AAA AGG TCA TCC AAG GAG GAA      2324
Thr Glu Thr Lys Glu Pro Lys Lys Ala Lys Arg Ser Ser Lys Glu Glu
        725                 730                 735

GCC GAG ATG GCC TAC AAA GAC TTC CTG CTC CAG TCC AGC ACC GTG GCC      2372
Ala Glu Met Ala Tyr Lys Asp Phe Leu Leu Gln Ser Ser Thr Val Ala
    740                 745                 750

GCC GAG GCC CAG GAC GGC CCC CAG GAA GCC TAG                          2405
Ala Glu Ala Gln Asp Gly Pro Gln Glu Ala
755                 760
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 764 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Leu Leu Phe Val Leu Thr Cys Leu Leu Ala Val Phe Pro Ala Ile
 1               5                  10                  15
```

Ser Thr Lys Ser Pro Ile Phe Gly Pro Glu Glu Val Asn Ser Val Glu
20 25 30

Gly Asn Ser Val Ser Ile Thr Cys Tyr Tyr Pro Pro Thr Ser Val Asn
35 40 45

Arg His Thr Arg Lys Tyr Trp Cys Arg Gln Gly Ala Arg Gly Gly Cys
50 55 60

Ile Thr Leu Ile Ser Ser Glu Gly Tyr Val Ser Ser Lys Tyr Ala Gly
65 70 75 80

Arg Ala Asn Leu Thr Asn Phe Pro Glu Asn Gly Thr Phe Val Val Asn
85 90 95

Ile Ala Gln Leu Ser Gln Asp Asp Ser Gly Arg Tyr Lys Cys Gly Leu
100 105 110

Gly Ile Asn Ser Arg Gly Leu Ser Phe Asp Val Ser Leu Glu Val Ser
115 120 125

Gln Gly Pro Gly Leu Leu Asn Asp Thr Lys Val Tyr Thr Val Asp Leu
130 135 140

Gly Arg Thr Val Thr Ile Asn Cys Pro Phe Lys Thr Glu Asn Ala Gln
145 150 155 160

Lys Arg Lys Ser Leu Tyr Lys Gln Ile Gly Leu Tyr Pro Val Leu Val
165 170 175

Ile Asp Ser Ser Gly Tyr Val Asn Pro Asn Tyr Thr Gly Arg Ile Arg
180 185 190

Leu Asp Ile Gln Gly Thr Gly Gln Leu Leu Phe Ser Val Val Ile Asn
195 200 205

Gln Leu Arg Leu Ser Asp Ala Gly Gln Tyr Leu Cys Gln Ala Gly Asp
210 215 220

Asp Ser Asn Ser Asn Lys Lys Asn Ala Asp Leu Gln Val Leu Lys Pro
225 230 235 240

Glu Pro Glu Leu Val Tyr Glu Asp Leu Arg Gly Ser Val Thr Phe His
245 250 255

Cys Ala Leu Gly Pro Glu Val Ala Asn Val Ala Lys Phe Leu Cys Arg
260 265 270

Gln Ser Ser Gly Glu Asn Cys Asp Val Val Val Asn Thr Leu Gly Lys
275 280 285

Arg Ala Pro Ala Phe Glu Gly Arg Ile Leu Leu Asn Pro Gln Asp Lys
290 295 300

Asp Gly Ser Phe Ser Val Val Ile Thr Gly Leu Arg Lys Glu Asp Ala
305 310 315 320

Gly Arg Tyr Leu Cys Gly Ala His Ser Asp Gly Gln Leu Gln Glu Gly
325 330 335

Ser Pro Ile Gln Ala Trp Gln Leu Phe Val Asn Glu Glu Ser Thr Ile
340 345 350

Pro Arg Ser Pro Thr Val Val Lys Gly Val Ala Gly Ser Ser Val Ala
355 360 365

Val Leu Cys Pro Tyr Asn Arg Lys Glu Ser Lys Ser Ile Lys Tyr Trp
370 375 380

Cys Leu Trp Glu Gly Ala Gln Asn Gly Arg Cys Pro Leu Leu Val Asp
385 390 395 400

Ser Glu Gly Trp Val Lys Ala Gln Tyr Glu Gly Arg Leu Ser Leu Leu
405 410 415

Glu Glu Pro Gly Asn Gly Thr Phe Thr Val Ile Leu Asn Gln Leu Thr
420 425 430

Ser Arg Asp Ala Gly Phe Tyr Trp Cys Leu Thr Asn Gly Asp Thr Leu
435 440 445

Trp Arg Thr Thr Val Glu Ile Lys Ile Ile Glu Gly Glu Pro Asn Leu
450 455 460

Lys Val Pro Gly Asn Val Thr Ala Val Leu Gly Glu Thr Leu Lys Val
465 470 475 480

Pro Cys His Phe Pro Cys Lys Phe Ser Ser Tyr Glu Lys Tyr Trp Cys
485 490 495

Lys Trp Asn Asn Thr Gly Cys Gln Ala Leu Pro Ser Gln Asp Glu Gly
500 505 510

Pro Ser Lys Ala Phe Val Asn Cys Asp Glu Asn Ser Arg Leu Val Ser
515 520 525

Leu Thr Leu Asn Leu Val Thr Arg Ala Asp Glu Gly Trp Tyr Trp Cys
530 535 540

Gly Val Lys Gln Gly His Phe Tyr Gly Glu Thr Ala Ala Val Tyr Val
545 550 555 560

Ala Val Glu Glu Arg Lys Ala Ala Gly Ser Arg Asp Val Ser Leu Ala
565 570 575

Lys Ala Asp Ala Ala Pro Asp Glu Lys Val Leu Asp Ser Gly Phe Arg
580 585 590

Glu Ile Glu Asn Lys Ala Ile Gln Asp Pro Arg Leu Phe Ala Glu Glu
595 600 605

Lys Ala Val Ala Asp Thr Arg Asp Gln Ala Asp Gly Ser Arg Ala Ser
610 615 620

Val Asp Ser Gly Ser Ser Glu Glu Gln Gly Gly Ser Ser Arg Ala Leu
625 630 635 640

Val Ser Thr Leu Val Pro Leu Gly Leu Val Leu Ala Val Gly Ala Val
645 650 655

Ala Val Gly Val Ala Arg Ala Arg His Arg Lys Asn Val Asp Arg Val
660 665 670

Ser Ile Arg Ser Tyr Arg Thr Asp Ile Ser Met Ser Asp Phe Glu Asn
675 680 685

Ser Arg Glu Phe Gly Ala Asn Asp Asn Met Gly Ala Ser Ser Ile Thr
690 695 700

Gln Glu Thr Ser Leu Gly Gly Lys Glu Glu Phe Val Ala Thr Thr Glu
705 710 715 720

Ser Thr Thr Glu Thr Lys Glu Pro Lys Lys Ala Lys Arg Ser Ser Lys
725 730 735

Glu Glu Ala Glu Met Ala Tyr Lys Asp Phe Leu Leu Gln Ser Ser Thr
740 745 750

Val Ala Ala Glu Ala Gln Asp Gly Pro Gln Glu Ala
755 760

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2031 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR-modified SEQ ID NO:1"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:111..2015
(D) OTHER INFORMATION:/product= "transcript from SC DNA fragment 1"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1..33
(D) OTHER INFORMATION:/product= "from PCR with 5' primer 1 (SEQ ID NO 5)"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:complement (1997..2031)
(D) OTHER INFORMATION:/product= "from PCR with 3' primer (SEQ ID NO 7)"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1..2031
(D) OTHER INFORMATION:/product= "SC DNA fragment 1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CGGTAGGCGT GTACGGTGGG AGGTCTATAT AGCAGAGCTC GTTTAGTGAA CCGTCAGAAT 60

TAATTCAGAT CTGGTACCAC GCGTATCGAT AAGCTTGAAT TCCACCAGCA ATGCTGCTCT 120

TCGTGCTCAC CTGCCTGCTG GCGGTCTTCC CAGCCATCTC CACGAAGAGT CCCATATTTG 180

GTCCCGAGGA GGTGAATAGT GTGGAAGGTA ACTCAGTGTC CATCACGTGC TACTACCCAC 240

CCACCTCTGT CAACCGGCAC ACCCGGAAGT ACTGGTGCCG GCAGGGAGCT AGAGGTGGCT 300

GCATAACCCT CATCTCCTCG GAGGGCTACG TCTCCAGCAA ATATGCAGGC AGGGCTAACC 360

TCACCAACTT CCCGGAGAAC GGCACATTCG TGGTGAACAT TGCCCAGCTG AGCCAGGATG 420

ACTCCGGGCG CTACAAGTGT GGCCTGGGCA TCAATAGCCG AGGCCTGTCC TTTGATGTCA 480

GCCTGGAGGT CAGCCAGGGT CCTGGGCTCC TAAATGACAC TAAAGTCTAC ACAGTGGACC 540

TGGGCAGAAC GGTGACCATC AACTGCCCTT TCAAGACTGA GAATGCTCAA AAGAGGAAGT 600

CCTTGTACAA GCAGATAGGC CTGTACCCTG TGCTGGTCAT CGACTCCAGT GGTTATGTGA 660

ATCCCAACTA TACAGGAAGA ATACGCCTTG ATATTCAGGG TACTGGCCAG TTACTGTTCA 720

GCGTTGTCAT CAACCAACTC AGGCTCAGCG ATGCTGGGCA GTATCTCTGC CAGGCTGGGG 780

ATGATTCCAA TAGTAATAAG AAGAATGCTG ACCTCCAAGT GCTAAAGCCC GAGCCCGAGC 840

TGGTTTATGA AGACCTGAGG GGCTCAGTGA CCTTCCACTG TGCCCTGGGC CCTGAGGTGG 900

CAAACGTGGC CAAATTTCTG TGCCGACAGA GCAGTGGGGA AAACTGTGAC GTGGTCGTCA 960

ACACCCTGGG GAAGAGGGCC CCAGCCTTTG AGGGCAGGAT CCTGCTCAAC CCCCAGGACA 1020

AGGATGGCTC ATTCAGTGTG GTGATCACAG GCCTGAGGAA GGAGGATGCA GGGCGCTACC 1080

TGTGTGGAGC CCATTCGGAT GGTCAGCTGC AGGAAGGCTC GCCTATCCAG GCCTGGCAAC 1140

TCTTCGTCAA TGAGGAGTCC ACGATTCCCC GCAGCCCCAC TGTGGTGAAG GGGGTGGCAG 1200

GAAGCTCTGT GGCCGTGCTC TGCCCCTACA ACCGTAAGGA AAGCAAAAGC ATCAAGTACT 1260

GGTGTCTCTG GGAAGGGGCC CAGAATGGCC GCTGCCCCCT GCTGGTGGAC AGCGAGGGGT 1320

GGGTTAAGGC CCAGTACGAG GGCCGCCTCT CCCTGCTGGA GGAGCCAGGC AACGGCACCT 1380

TCACTGTCAT CCTCAACCAG CTCACCAGCC GGGACGCCGG CTTCTACTGG TGTCTGACCA 1440

ACGGCGATAC TCTCTGGAGG ACCACCGTGG AGATCAAGAT TATCGAAGGA GAACCAAACC 1500

TCAAGGTACC AGGGAATGTC ACGGCTGTGC TGGGAGAGAC TCTCAAGGTC CCCTGTCACT 1560

TTCCATGCAA ATTCTCCTCG TACGAGAAAT ACTGGTGCAA GTGGAATAAC ACGGGCTGCC 1620

AGGCCCTGCC CAGCCAAGAC GAAGGCCCCA GCAAGGCCTT CGTGAACTGT GACGAGAACA 1680

GCCGGCTTGT CTCCCTGACC CTGAACCTGG TGACCAGGGC TGATGAGGGC TGGTACTGGT 1740

GTGGAGTGAA GCAGGGCCAC TTCTATGGAG AGACTGCAGC CGTCTATGTG GCAGTTGAAG 1800

AGAGGAAGGC AGCGGGGTCC CGCGATGTCA GCCTAGCGAA GGCAGACGCT GCTCCTGATG 1860

AGAAGGTGCT AGACTCTGGT TTTCGGGAGA TTGAGAACAA AGCCATTCAG GATCCCAGGC 1920

TTTTTGCAGA GGAAAAGGCG GTGGCAGATA CAAGAGATCA AGCCGATGGG AGCAGAGCAT 1980

CTGTGGATTC CGGCAGCTCT GAGGAACAAG GTGGATGATC TAGAGCGCTG G 2031

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1936 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR-modified SEQ ID NO:1"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:16..1920
(D) OTHER INFORMATION:/product= "transcript from SC DNA fragment 2"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1..46
(D) OTHER INFORMATION:/product= "from PCR with 5' primer 2 (SEQ ID NO 6)"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:complement (1902..1936)
(D) OTHER INFORMATION:/product= "from PCR with 3' primer (SEQ ID NO 7)"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1..1936
(D) OTHER INFORMATION:/product= "SC DNA fragment 2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GGGAAGCTTG CCACCATGGT GCTCTTCGTG CTCACCTGCC TGCTGGCGGT CTTCCCAGCC 60

ATCTCCACGA AGAGTCCCAT ATTTGGTCCC GAGGAGGTGA ATAGTGTGGA AGGTAACTCA 120

GTGTCCATCA CGTGCTACTA CCCACCCACC TCTGTCAACC GGCACACCCG GAAGTACTGG 180

TGCCGGCAGG GAGCTAGAGG TGGCTGCATA ACCCTCATCT CCTCGGAGGG CTACGTCTCC 240

AGCAAATATG CAGGCAGGGC TAACCTCACC AACTTCCCGG AGAACGGCAC ATTCGTGGTG 300

AACATTGCCC AGCTGAGCCA GGATGACTCC GGGCGCTACA AGTGTGGCCT GGGCATCAAT 360

AGCCGAGGCC TGTCCTTTGA TGTCAGCCTG GAGGTCAGCC AGGGTCCTGG GCTCCTAAAT 420

GACACTAAAG TCTACACAGT GGACCTGGGC AGAACGGTGA CCATCAACTG CCCTTTCAAG 480

ACTGAGAATG CTCAAAAGAG GAAGTCCTTG TACAAGCAGA TAGGCCTGTA CCCTGTGCTG 540

GTCATCGACT CCAGTGGTTA TGTGAATCCC AACTATACAG GAAGAATACG CCTTGATATT 600

CAGGGTACTG GCCAGTTACT GTTCAGCGTT GTCATCAACC AACTCAGGCT CAGCGATGCT 660

GGGCAGTATC TCTGCCAGGC TGGGGATGAT TCCAATAGTA ATAAGAAGAA TGCTGACCTC 720

CAAGTGCTAA AGCCCGAGCC CGAGCTGGTT TATGAAGACC TGAGGGGCTC AGTGACCTTC 780

CACTGTGCCC TGGGCCCTGA GGTGGCAAAC GTGGCCAAAT TTCTGTGCCG ACAGAGCAGT 840

GGGGAAAACT GTGACGTGGT CGTCAACACC CTGGGGAAGA GGGCCCCAGC CTTTGAGGGC 900

AGGATCCTGC TCAACCCCCA GGACAAGGAT GGCTCATTCA GTGTGGTGAT CACAGGCCTG 960

AGGAAGGAGG ATGCAGGGCG CTACCTGTGT GGAGCCCATT CGGATGGTCA GCTGCAGGAA 1020

GGCTCGCCTA TCCAGGCCTG GCAACTCTTC GTCAATGAGG AGTCCACGAT TCCCCGCAGC 1080

CCCACTGTGG TGAAGGGGGT GGCAGGAAGC TCTGTGGCCG TGCTCTGCCC CTACAACCGT 1140

AAGGAAAGCA AAAGCATCAA GTACTGGTGT CTCTGGGAAG GGGCCCAGAA TGGCCGCTGC 1200

CCCCTGCTGG TGGACAGCGA GGGGTGGGTT AAGGCCCAGT ACGAGGGCCG CCTCTCCCTG 1260

CTGGAGGAGC CAGGCAACGG CACCTTCACT GTCATCCTCA ACCAGCTCAC CAGCCGGGAC 1320

GCCGGCTTCT ACTGGTGTCT GACCAACGGC GATACTCTCT GGAGGACCAC CGTGGAGATC 1380

AAGATTATCG AAGGAGAACC AAACCTCAAG GTACCAGGGA ATGTCACGGC TGTGCTGGGA 1440

GAGACTCTCA AGGTCCCCTG TCACTTTCCA TGCAAATTCT CCTCGTACGA GAAATACTGG 1500

TGCAAGTGGA ATAACACGGG CTGCCAGGCC CTGCCCAGCC AAGACGAAGG CCCCAGCAAG 1560

GCCTTCGTGA ACTGTGACGA GAACAGCCGG CTTGTCTCCC TGACCCTGAA CCTGGTGACC 1620

AGGGCTGATG AGGGCTGGTA CTGGTGTGGA GTGAAGCAGG GCCACTTCTA TGGAGAGACT 1680

GCAGCCGTCT ATGTGGCAGT TGAAGAGAGG AAGGCAGCGG GGTCCCGCGA TGTCAGCCTA 1740

GCGAAGGCAG ACGCTGCTCC TGATGAGAAG GTGCTAGACT CTGGTTTTCG GGAGATTGAG 1800

AACAAAGCCA TTCAGGATCC CAGGCTTTTT GCAGAGGAAA AGGCGGTGGC AGATACAAGA 1860

GATCAAGCCG ATGGGAGCAG AGCATCTGTG GATTCCGGCA GCTCTGAGGA ACAAGGTGGA 1920

TGATCTAGAG CGCTGG 1936

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CGGTAGGCGT GTACGGTGGG AGGTCTATAT AGC 33

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGGAAAGCTT CCACCATGGT GCTCTTCGTG CTCACCTGCC TGCTGG 46

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CCAGCGCTCT AGATCATCCA CCTTGTTGTT CCTCAGAG 38

CLAIMS:

1. Recombinant secretory component (rSC) or a functional fragment thereof, obtainable by a process comprising culturing a CHO SSF 3 cell transfected with a vector comprising a DNA coding for said secretory component or a fragment thereof, and isolating the expressed protein from the culture medium.

2. Secretory component according to claim 1 having a content of N-glycolylneuraminic acid that is below 0.5% in respect to total sialic acid.

3. Process for the production of a secretory component or a functional fragment thereof according to claim 1 comprising
   a) constructing a vector capable of expressing the secretory component or a functional fragment thereof;
   b) transfecting a CHO SSF 3 cell with said vector;
   c) culturing the transfected cells; and
   d) isolating the secretory component or a fragment thereof from the culture medium.

4. Process according to claim 3, wherein the vector is capable of expressing a soluble fragment of the secretory component.

5. Process according to claim 3, wherein the vector comprises a functional fragment of the secretory component as depicted in SEQ ID NO 3 or SEQ ID NO 4.

6. Process according to claim 3, wherein the vector is a mammalian cell expression vector.

7. Process according to claim 3, wherein the vector is based on pCB6 or pCGA93D-PPREN.

8. Process according to claim 3, wherein the vector is pCB6-SC, pMC-SC or pCGA93D-SC.

9. Process according to claim 3, wherein the vector integrates into the chromosome of the CHO SSF 3 cells.

10. Process according to claim 9, wherein transfected CHO SSF 3 cells with amplified integrated vector DNA are selected.

11. Process according to claim 3, wherein the cells are cultured in serum-free medium.

12. Process according to claim 3, wherein Pluronic® is added to the culture medium to increase productivity.

13. Method for the crystallization of a secretory component or a functional fragment thereof according to claim 1, comprising placing a solution of said secretory component or a functional fragment thereof in a vessel containing a precipitating agent buffer, wherein the solution and the buffer are separated.

14. Method according to claim 13, wherein the solution of said secretory component or the functional fragment thereof contains precipitating agent buffer.

15. Method according to claim 13, wherein the precipitating agent buffer comprises Na-citrate, HEPES, NaN_3, ammonium phosphate and/or Li_2SO_4.

16. Method according to claim 13, wherein the precipitating agent buffer comprises Na-citrate, NaN_3 and ammonium phosphate; or HEPES, NaN_3 and Li_2SO_4.

17. Method according to claim 13, wherein said secretory component or the functional fragment thereof is placed in a hanging manner over the precipitating agent buffer.

18. Use of rSC according to claim 1 in a method of treatment.

19. Use of the crystallized rSC according to claim 1 for the determination of the 3-dimensional structure.

20. Use of rSC according to claim 1 for binding studies.

21. Use of rSC according to claim 1 in the screening of compounds for their ability to influence poly-Ig binding.

22. Use of rSC according to claim 1 for the identification of antagonists or agonists of the SC mediated IgA transport.

23. A modified rSC according to claim 1 that is immobilized on a solid carrier or that bears one or more identifiable maker like biotin or a radioactive, fluorescent or chemoluminescent group.

24. Use of an antagonist or agonist identified using rSC according to claim 1, in a method of treatment.

25. Use of an antagonist or agonist identified using the 3-dimensional structure according to claim 19, in a method of treatment.

INTERNATIONAL SEARCH REPORT

Int onal Application No
PCI/EP 95/04797

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 C07K1/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EXPERIENTIA, vol. 50, BASEL CH, page A27 XP000567378 S.COTTET ET AL.: "Use of Vaccinia virus recombinants to produce secretory component" Abstract S08-06 ---	1,2
Y	HUMAN GENETICS, vol. 87, no. 6, 1991, pages 642-648, XP000567174 P.KRAJCI ET AL.: "The human transmembrane secretory component (poly-Ig receptor): molecular cloning, restriction fragment polymorphism and chromosomal sublocalization" see figure 2 --- -/--	3-12

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 April 1996	09.05.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Cupido, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCI/EP 95/04797

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF BIOTECHNOLOGY, vol. 32, no. 1, 15 January 1994, AMSTERDAM NL, pages 191-202, XP002000565 F.ASSELBERGS ET AL.: "Scaled-up production of recombinant human renin in CHO cells for enzymatic and X-ray structure analysis" see page 193, column 1, paragraph 2 ---	3-12
X	BEHRING INSTITUTE MITTEILUNGEN, vol. 54, June 1974, pages 9-21, XP000567333 H.HAUPT AND S.BAUDNER: "Isolierung, Kristallisation und Eigenschaften der freien Sekretorischen Komponente aus Human-Kolostrum" see paragraph bridging pages 11 and 12 ---	1,13,14
A	C.R.GANDOR: Establishment and characterization of growth-factor prototropic CHO cell lines for the production of recombinant proteins. Doctoral thesis, Swiss XP002000666 Federal Institute of Technology, Zürich 1993. -----	1,3,6,11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 95/04797

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remarks: Although claims 18,24 and 25 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.